CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
I. STUDY IDENTIFICATION

Active Ingredient: North American P1/P13 Creosote CTM (p. 133)
Chemical Code #: 171 **ID #:** SBC-154344-E
Document #: 50436-025 **Record #:** 138222
EPA Reg. #: 61468-0- **SB 950 #:** 157
Study Type: Reproduction, rat
Full Study Title: "Two generation reproduction/fertility study in rats"
Company Sponsor: Koppers Industries, Inc.
Conducting Laboratory: IRDC **Final Report Date:** 3/13/95

II. SUMMARY OF WORKSHEET

A. **STUDY STATUS: Is report complete?** - yes
Is study acceptable? - yes
yes - **Minor variances from guidelines** - **Insufficient data**

B. **CONCLUSIONS: Does this study as reported demonstrate a possible adverse health effect?:** yes (low pregnancy indices, decreased numbers of live pups/litter, increased numbers of stillborn pups).

C. **ONE LINER - Summary of the study:**
50436-025 138222 York, R.G., "Two generation reproduction/fertility study in rats", IRDC Lab. Project ID #672-006, 3/13/95. Charles River Crl:CD* VAF* rats, 26/sex/group, were dosed with "North American P1/P13 Creosote CTM" (a representative commercial composite) in corn oil vehicle by daily gavage at 0, 25, 75, or 150 mg/kg/day. This was a typical reproduction study with 1 litter per generation, unusual in that pre-mating period dosing of F1 rats was delayed until 35 days of age, and the pre-mating treatment phase lasted about 17 weeks. Parental effects NOEL < 25 mg/kg/day (decrement in pre-mating body weights, F1 females). Common parental effects at 75 to 150 mg/kg/day included minor body weight decrements and clinical signs of increased salivation and anogenital staining. At 150 mg/kg/day, body weight decrements were marked, especially for F1 males. Reproductive effects NOEL < 25 mg/kg/day (very low fertility and pregnancy indices, without dose-response, in the F1 parental generation). At 75 to 150 mg/kg/day there was a decrease in live pups per litter. This was associated with increases in stillborn pups at 75 mg/kg/day, yet a much greater increase in stillborn pups at 150 mg/kg/day did not fully account for the dramatic drop in live pups born at that dose. Gestation length was slightly protracted at 150 mg/kg/day. Developmental toxicity NOEL = 25 mg/kg/day (modest pup b.w. decrements during lactation). Pup survival at 150 mg/kg/day was reduced in the F0 mating trial. There was a notable incidence of microphthalmia among F1 pups at 150 mg/kg/day (5 pups from 2 litters). Study is **acceptable, with "**possible adverse effects**" (low pregnancy indices at all dose levels in F1 mating trial, decreased live pups/litter, at least partially due to stillborn pups). C. Aldous, 7/20/95.**

Staff Toxicologist

July 25, 1995
Date

III. PROTOCOL SUMMARY

A. **ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:**
Species: rat
Strain: Charles River Crl:CD® VAF® rats
Source of animals: Charles River Laboratories, Portage MI
Age at start: 46 days (p. 18)
Route of administration: gavage, as a single daily dose.
Vehicle: Corn oil. Volume was 10 ml/kg, as stated on p. 11, rather than a "constant volume of 10.0 mg/kg/day", as mistakenly stated on p. 18.
Period of treatment: From age 46 days for 8 wk prior to mating of F0 rats, and continually through the lactation period. F1 rats were dosed beginning at 35 days of age (to prevent losses to gavage errors on small, active weanlings). These F1 parental animals were treated for at least 113 days prior to mating, and also on through the lactation period. (See p. 18).
Study dates: First treatment on March 8, 1993. Study termination on Jan. 5, 1994 (pp. 14-15).

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. **STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).**

1. **Test article (assay, purity, lot #, stability):** Test article was "North American P1/P13 Creosote CTM". It was given a "Pro. Number" designation of KTOR-247552-3 (p. 133). Note that the concurrent teratology study designated the test article as "TOR-247552-3" (p. 45 of Document No. 50436-024): these are likely to be the same material. As noted in the teratology study review, analyses were performed by assaying for the "9 Most Prevalent Compounds in Creosote" by glc (pp. 139 ff. in this volume). OK.
* 2. **Analysis of dosing material (stability, homogeneity, compound content):** Content of high dose material on day 1 was 19% higher than nominal (p. 61). Assay for content was evidently only performed for this study one further time (on week 2, p. 61). There were no other instances of assayed levels differing from target by more than 9% (p. 63). As expected, stability was acceptable (p. 62). Homogeneity of formulated material was satisfactory (p. 61). Failure to periodically assay the test article solutions or suspensions throughout the duration of the study is a notable deficiency, but does not invalidate the study. Test material was prepared weekly and stored at RT (p.18).
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** OK
6. **Number of animals (start and termination):** OK. In order to ensure that intubation errors did not limit the numbers of F1 parents, two precautions were taken (pp. 18, 19). First, dosing began at day 35 instead of immediately at weaning. Second, 5 extra rats/sex/group were gavaged for one week in case of gavage-error deaths among the designated 26/sex/group for F1 parents. It is not evident whether any of the original 26/sex/group needed replacement, however there was no indication of attrition early in the dosing period for the F1 rats (see especially pp. 277 ff.), suggesting that there were few if any losses due to gavage error during the first week of F1 dosing.
7. **Randomization of animals:** OK
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK

10. **Exposure conditions (schedule and methods):** OK (see II.A., above).
11. **Controls (negative and positive):** OK
12. **Observations (cageside, body weight, physicals, etc):** OK
* 13. **Necropsies (required animals, tissues, or parameters):** Report states that uteri of dams which appeared nongravid were stained with ammonium chloride solution (p. 21), citing the same reference of Kopf, Lorenz, and Salewski (1964) which was cited in the corresponding IRDC rat teratology study (Record No. 138221). The teratology study indicated that implantation sites were visualized by ammonium sulfide solution (p. 15 of that record), which is consistent with standard practices. Page 42 of the present report states that ammonium sulfide is used in this method. Evidently this study used ammonium sulfide and mistakenly named the wrong salt in the report.
14. **Histopathology (tissues, groups, and number of animals):** Reproductive tissues (see p. 405) were examined for control and high dose groups. Gross lesions were also microscopically examined (p. 22). OK.
15. **Fetal examination:** Weanlings (other than F1 parental rats) were examined grossly and discarded (p. 21), as were day-4 culled pups (p. 20). Also, pups dying on study were examined to the extent possible (i.e., when not prevented by cannibalism or autolysis, p. 20). Individual data were limited to pups with noteworthy gross findings. These data, as presented (see for instance pp. 368 ff.), did not disclose ages of pups at death. Nevertheless, pups noted to be "not tattooed" might be presumed to have died very early, perhaps before being seen alive. This was the case with the 5 high dose pups having microphthalmia (p. 371). OK.
16. **Appropriateness of methods:** None of the problems noted above would invalidate the study. OK.
17. **Treatment of results (data summarization and statistics):** OK
* 18. **Study report (complete, reflects data, data cited but missing):** Generally OK, however individual clinical signs observations in Appendix D are consistent with the summary table on p. 27, but not with the more extensive summary tables on pp. 64 ff. All discrepancies are small, and none would affect study interpretation.
19. **Consistency (with other studies of this type):** OK
20. **Good laboratory practice (internal audits, sign-offs):** OK

V. RESULTS

A. **EFFECTS REPORTED:** [NOTE: this study was not flagged for potential adverse effects by company representatives (see p. 0004)]. Flagging criteria are tied to the ADI, which is out of the scope of this review.

There was appreciable mortality in various treatment groups, largely due to gavage errors. None of the deaths were definitively due to test article. Below is an enumeration of mortalities, including where possible an assessment of causes of deaths (from pp. 25-27).

Mortality of Parental Animals

	Dose (mg/kg/day)							
	Males				Females			
	0	25	75	150	0	25	75	150
FO parental rats N =	(26)	(26)	(26)	(26)	(26)	(26)	(26)	(26)
Total # Deaths	1	0	1	1	1	2	4	4
Probable gavage errors	1	-	-	-	1	2	3	3
Cause evident: not related to test article	-	-	-	1	-	-	-	-
Cause unknown: not presumed to be treatment effect	-	-	1	-	-	-	1	-
Probably test article-related	-	-	-	-	-	-	-	1†
F1 parental rats N =	(26)	(25)	(26)	(26)	(26)	(27)	(26)	(26)
Total # Deaths	4	2	0	2	0	1	3	5
Probable gavage errors	4	2	-	2	-	1	3	2
Cause evident: not related to test article	-	-	-	-	-	-	-	-
Cause unknown: not presumed to be treatment effect	-	-	-	-	-	-	-	3
Probably test article-related	-	-	-	-	-	-	-	-

† *Dam with 7 stillborns had clinical signs typical of test article response. Dam may have died resulting from creosote, based on clinical signs, although not specifically stated as such by investigators (p. 25).*

The more marked or characteristic in-life observations follow. At 75 mg/kg/day, "Increased salivation" was generally evident, and "anogenital staining" was appreciably elevated in both sexes in the F1 generation.

In-life Observations (p. 27, pp. 64 ff.)

	Dose (mg/kg/day)							
	Males				Females			
	0	25	75	150	0	25	75	150
FO parental rats N =	(26)	(26)	(26)	(26)	(26)	(26)	(26)	(26)
Increased salivation	0	0	0	6	0	0	4	5
Discolored urine	0	1	0	2	0	0	1	3
Anogenital staining	9	9	13	15	6	1	7	23
F1 parental rats N =	(26)	(25)	(26)	(26)	(26)	(27)	(26)	(26)
Increased salivation	0	1	6	3	0	1	7	10
Discolored urine	1	2	0	6	0	0	1	1
Anogenital staining	9	5	17	23	1	2	10	22
Impaired limb function	0	0	0	4	0	0	2	2
Body surface stained	3	2	2	11	3	1	5	8

Body weights during the premating periods demonstrated that there were treatment effects, however intermediate dose effects were sometimes poorly defined (pp. 68, 69, 74, and 75).

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Body Weights (g) During Premating Periods

	Males				Females			
	0	25	75	150	0	25	75	150
Study Week				F0 parental rats				
0	193	191	194	194	142	143	140	138
1	250	247	250	249	168	167	163	164
4	373	365	367	360	214	209	216	207
8	480	461	464	447**	251	245	250	247
12	524	504	503	485*	N/A†	N/A	N/A	N/A
15	552	531	532	502**	N/A	N/A	N/A	N/A
Week of Age				F1 parental rats				
4	98	93	86**	80**	93	88	79**	72**
8	318	304	284**	267**	204	192*	190*	177**
12	453	430	402**	369**	247	233*	238	223**
16	527	498*	466**	411**	272	253**	260	241**
20	564	531	487**	423**	285	266**	270*	250**
24	583	531*	490**	439**	325	284*	279**	271**
28	611	557	510**	453**	N/A	N/A	N/A	N/A

*, ** Significantly different from controls, $p < 0.05$ and $p < 0.01$, respectively.

N/A Indicates very small N values, such that mean values are of questionable statistical significance.

There were no statistically significant differences between body weights of F0 females during gestation (p. 70), although body weight changes in the two higher dose groups were significantly reduced (p. 71). The latter change reflects in part the smaller litter sizes in the higher two groups (p. 89), hence may not reflect primary effects on the dams. The 150 mg/kg/day female body weights also fell significantly below other groups at days 7 and 14 of lactation (p. 72). The extent to which the latter differences also arose from markedly reduced litter sizes cannot be determined.

Similar patterns of maternal weight during gestation and lactation were observed for the F1 dams, a major difference being the substantial body weight decrements of the high dose females at the beginning of gestation (p. 76).

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Gestation and Lactation Body Weights (g)
(from pp. 70, 72, 76, and 78)

	F0 Females				F1 Females			
	0	25	75	150	0	25	75	150
Gestation Day								
0	247	253	254	248	292	272	283	249**
6	268	269	270	268	312	292	301	266**
15	304	305	303	304	338	316	323	289**
20	371	365	359	352	394	373	366	317**
Lactation Day								
0	282	281	278	274	319	293	281	274**
7	300	296	290	282*	325	305	282*	278**
14	320	318	313	297**	333	319	304*	278**
21	310	307	306	300	324	309	306	276**

*, ** Significantly different from controls, $p < 0.05$ and $p < 0.01$, respectively.

Food consumption decrements (units of g/rat/day) during pre-mating periods were either non-existent (pp. 80 and 81 for F0 rats), or were limited to the higher two dose groups during the first 2 to 5 weeks (F1 rats, pp. 84 to 85). There were no consistent patterns of food consumption changes during gestation (pp. 82 to 86). Nevertheless, consistently low food consumption was recorded during lactation in both generations, reflecting at least in part the lesser demand for milk due to small litter sizes (pp. 83 and 87).

	F0 Females				F1 Females			
	0	25	75	150	0	25	75	150
Lactation Days								
0-7	29	27	24	19**	20	23	22	14*
7-14	47	43	40	29**	36	41	32	16**
14-21	62	54*	51*	35**	45	52	42	17**

*, ** Significantly different from controls, $p < 0.05$ and $p < 0.01$, respectively.

There were several reproductive effects with clearly definitive effects at the upper one or two dose levels. These included great reductions in live pups per litter, explained only partially by dramatic increases in stillborn pups at the high dose level. Neonatal viability for the first few days after birth was also reduced at 150 mg/kg/day. Meaningful pup body weight gain decrements appeared to have an LEL of 75 mg/kg/day, considering both generations. (The 25 mg/kg/day F1 pups were significantly lower in body weight than F1 controls, however the 25 mg/kg/day F2 pups were slightly higher in b.w. than the F2 controls). Longer gestation duration at 150 mg/kg/day appears to be a treatment effect, and could have contributed to pup mortality around time of parturition. The surprisingly low pregnancy indices for all treated groups (without dose-response relationship) are discussed in section IV.B. of this review.

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FO Reproduction Parameters; also Offspring Viability and Growth

		Dose (mg/kg/day)			
		0	25	75	150
# FO females on study	(Page 4) (17)	26	26	26	26
# FO males on study	(17)	26	26	26	26
# FO males FD + KE prior to mating	(25, 347 ff)	1	0	0	0
# FO females FD + KE prior to mating	(347 ff)	0	0	2	0
# FO females mated	(88)	25	26	24	26
# FO females pregnant	(88)	23	20	20	22
# FO females dying during gestation	(347 ff)	1	1	1	1
Gestation length (days)	(88)	22.1	22.4	22.4	22.9
# FO live litters born	(91, 351 ff)	22	18	18[1]	17
# FO litters with live pups on day 21	(91, 351 ff)	22	17	17	15[2]
# FO still-born litters	(351 ff)	0	1	0	4
Mean dead pups/litter on day 0	(89)	0.2	0.1	0.4	2.4*
Mean live pups per litter[3] (day 0)	(89)	13.0	12.2	11.1**	6.8**
Offspring viability:					
% born alive	(90)	99	99	96	74**
% liveborn living to day 4	(90)	98	94	85	77**
% of day 4 post-cull alive at day 7	(90)	100	99	99	99
% of day 7 pups alive at day 14	(90)	100	99	99	99
% of day 14 pups alive at day 21	(90)	100	100	100	100
Offspring growth: mean pup b.w. (g)					
Day 0	(91)	6.1	6.0	5.7	5.9
Day 4 (post-culling)	(91)	9.8	9.3	9.0	8.8
Day 7	(91)	16.3	15.1	13.9*	12.6*
Day 14	(91)	34.2	31.0**	29.0**	24.8**
Day 21 - Male	(91)	56.4	51.4**	46.3**	39.8**
Day 21 - Female	(91)	53.6	49.9*	44.7**	39.0**

[1] One 75 mg/kg/day dam died during delivery on day 24 p.c. (pp. 349, 353).
[2] Page 91 states 14 remaining litters in high dose group on day 21, but page 354 (offspring viability individual data) lists male or female survivors in 15 litters. The latter number was used. One of the two litter losses was due to death of Dam #47315 due to apparent gavage error (pp. 25, 354).
*, ** Significantly different from controls, $p < 0.05$ and $p < 0.01$, respectively.
[3] Presented as live pups per litter delivered. Any dam with offspring (dead or alive) counts as a "litter" for this statistic.

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F1 Reproduction Parameters; also Offspring Viability and Growth

	(Page #)	Dose (mg/kg/day) 0	25	75	150
# F1 males on study	(66)	26	25	26	26
# F1 females on study	(67)	26	27	26	26
# F1 males FD + KE prior to mating[1]	(26, 373 ff)	2	1	0	1
# F1 females FD + KE prior to mating	(26-27, 373 ff)	0	0	1	3
# F1 females mated	(96)	24	23	15	20
Female copulatory index (# mated/# paired)	(96)	92	85	60**	87
# F1 females pregnant	(96)	16	7	6[2]	11
# F1 females dying during gestation[3]	(373 ff)	0	1	0	1[4]
Gestation length (days)	(96)	22.4	22.3	22.8	23.4
# F1 live litters born	(99, 377 ff)	16	7	5	4
# F1 litters with living pups on day 21	(99)	14	6	4	4
# F1 still-born litters	(377 ff)	0	0	1	5
Mean dead pups/litter on day 0	(97)	0.3	0.6	2.7	1.7
Mean live pups per litter[5] (day 0)	(97)	9.6	11.1	7.3	1.8*
Offspring viability:					
% born alive	(98)	97	95	73*	52*
% liveborn living to day 4	(98)	86	81	100	88
% of day 4 post-cull alive at day 7	(98)	100	100	100	93
% of day 7 pups alive at day 14	(98)	100	100	100	100
% of day 14 pups alive at day 21	(98)	100	100	88	100
Offspring growth: mean pup b.w. (g)	(99)				
Day 0		6.2	5.8	6.0	6.2
Day 4 (post-culling)		9.6	10.1	9.1	9.1
Day 7		15.4	16.2	14.1	13.2
Day 14		31.0	32.6	27.6	23.9*
Day 21 - Male		51.1	53.3	46.1	39.3
Day 21 - Female		48.4	51.1	44.5	38.0*

[1] Death of male was presumed prior to pairing if the respective ID# was not found as "first male used" in Appendix K.
[2] Summary table, p. 96, says 7 gravid females, however only 6 can be located in individual data on p. 375 (5 which delivered, and 1 gravid dam which did not).
[3] Or presumed gestation if death occurred too shortly after copulation to confirm pregnancy.
[4] Of 11 pregnant high dose F1 dams, one died on gestation day 25, and one "did not deliver" (p. 376).
[5] Presented as live pups per litter delivered. Any dam with offspring (dead or alive) counts as a "litter" for this statistic.
* Significantly different from controls, $p < 0.05$.

A summary of F1 offspring antemortem observations is presented on pp. 92-94, which confirms the expected (i.e. there were more offspring found dead or missing in the higher dose groups). The necropsy data for F1 pups (p. 95), however, are consistent with the recent IRDC teratology study in that microphthalmia was seen in high dose pups more frequently than normally expected. Numbers of pups per group were not given in the summary table, but the table includes pups which died or were killed on days 4, 21, or 42. The NOEL from this study is 75 mg/kg/day, since a single incident such as was seen at 25 mg/kg/day is well within historical incidence range (see review for IRDC Lab ID 671-020, DPR Record No. 138221). The NOEL for microphthalmia in that study was 25 mg/kg/day.

Microphthalmia in F1 Pups at Necropsy

	Dose (mg/kg/day)			
	0	25	75	150
Microphthalmia incidence [fetal, (litter)]	0	1 (1)	0	5 (2)

There were no unique antemortem or necropsy observations for F2 offspring, however numbers of observable pups were very small in all F2 treatment groups (pp. 100-104).

A presentation of numbers of implantation sites less numbers of recorded offspring delivered, yielding postimplantation loss and/or cannibalization, highlights high dose effects from the perspective of maternal necropsy data (pp. 104, 105). These data demonstrate that it is primarily at some phase(s) of postimplantation that the marked treatment effects occur.

Uterine Observations

	F0 Dams				F1 Dams			
	0	25	75	150	0	25	75	150
# Implantation sites	14.0	13.4	13.2	12.5	11.5	12.3	12.5	9.4
# Offspring observed	13.2	12.3	11.5	9.2	9.9	11.7	10.0	3.4
(Difference)	0.8	1.1	1.8	3.5	1.6	0.6	2.5	6.0

Overall macroscopic observations of parental F0 or F1 rats did not indicate compound-related effects (pp. 106 ff). Microscopic observations were similarly not remarkable (pp. 121 ff).

B. **NO OBSERVED EFFECT LEVEL (NOEL):** Parental effects NOEL < 25 mg/kg/day (decrement in pre-mating body weights, F1 females). Reproductive effects NOEL < 25 mg/kg/day (very low pregnancy indices without dose-response in the F1 parental generation). Developmental toxicity NOEL = 25 mg/kg/day (modest pup b.w. decrements during lactation).

VI. DISCUSSION

A. **MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific:** Study meets minimal criteria for acceptability. The lack of a definitive reproductive effects NOEL is a significant weakness in this study, especially since there is no dose-response relationship for reduced fertility and pregnancy indices.

B. **DISCUSSION OF RESULTS (if necessary). Were there possible adverse health effects? Are there any recommendations specific to this study?**

There is no NOEL established for reproductive effects. Prenatal death was clearly evident at 150 mg/kg/day, as indicated by numbers of stillborn litters. Mean live litter sizes were smaller at 75 and 150 mg/kg/day in both generations (not statistically significantly at 75 mg/kg/day in the second generation, but nevertheless plausibly treatment-related). The mean number of dead F2 offspring on lactation day 0 was elevated for both 75 and 150 mg/kg/day groups, such that the percent of pups born alive was significantly reduced in both groups in dose-related fashion. Pup deaths in the F1 litters

at 150 mg/kg/day were also increased during the timeframe of 0 to 4 days postpartum. This was not observed in the F2 generation, however there were only 16 pups (4 litters) alive at day 0: too few pups to evaluate trends in pup mortality.

The most puzzling finding was very low pregnancy indices (# pregnant/ # mating) levels in all treated F1 dams, without dose-response relationship. There was also an unusually low "copulatory index" (# females mated/# paired) for the 75 mg/kg/day group, which was considered by investigators to be a treatment effect (p. 31). The low fertility indices (# pregnant/# paired) in all F1 pup treatment groups were also considered by investigators to be a treatment effect (p. 31), nevertheless the primary reproductive failure in this generation was not failure to mate, but failure of mated females to become pregnant. Investigators noted that CD females suffer from subfertility problems as they become larger, and that F1 females in this study were into that critical range (greater than 310 g). Investigators state that "The long premating exposure necessitated by this EPA-mandated protocol permit the females to be excessively heavy by the time of mating, especially the F1 generation" (p. 37). Summary body weight data for F1 females (p. 75) shows that females were about 17 weeks on treatment before pairing, i.e., they were 5 weeks old at onset of dosing (see p. 18), with pairing at about 22 weeks of age (when the "N" values on p. 75 dropped sharply, denoting the end of the pre-mating period). If rats had been paired at week 14 of dosing, as suggested in the 1982 and 1984 FIFRA guidelines, the heaviest group (controls) would have had mean weight of about 285 g, well below the critical weight for subfertility problems cited above. Thus it appears that overweight dams might not be the basis of poor fertility. In the absence of any more definitive explanation, this reviewer agrees that the pregnancy and fertility indices should be treated as having no NOEL over the dose range of this study, even though there is a puzzling lack of dose-response relationship.